Isolation of novel temperate bacteriophages from a new subspecies of *Desulfovibrio*

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Summary

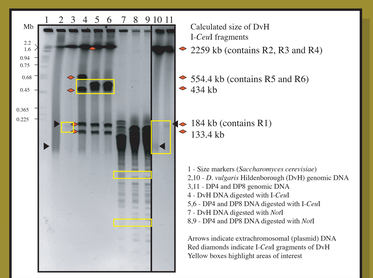
Nine *Desulfovibrio vulgaris*-like bacteria (DP1-9) were isolated from a heavy metal impacted field site (Lake DePue, Illinois) using lactate as a carbon source and sulfate as an electron acceptor. All had identical 16S rRNA, intergenic transcribed spacer (ITS) regions and *dsrAB* genes that were 99% identical to the orthologous genes of *D. vulgaris* Hildenborough (DvH). Their growth rates at different temperatures on B3 medium using lactate as a carbon source and sulfate as an electron acceptor were comparable. Pulse field gel electrophoretic analysis of I-*Ceu*I whole genome digests identified a large deletion in the genomes of all isolates. Complementary whole-genome microarray hybridization revealed that approximately 300 deleted genes were absent in the genome of the Lake DePue isolate and distributed in six chromosomal regions. These genes have been annotated as conserved, conserved hypothetical or phage-related genes in *D. vulgaris* Hildenborough. The absence of these genes in the Lake DePue isolate was confirmed through PCR analysis using primers complementary to regions flanking the "phage-regions". Sequencing of these regions revealed areas of high nucleotide similarity when compared with the Hildenborough strain, as well as areas of low nucleotide similarity. Genomic rearrangement was also observed in one of the regions. Suppressive subtractive hybridization (SSH) further suggested the presence of novel genetic material within the Lake DePue isolates. The close phylogenetic relationship, coupled with the observed genetic variation, allowed the Lake DePue isolate to serve as a host for latent viruses of *D. vulgaris* Hildenborough. Two distinct phage morphotypes have so far been identified by electron microscopy. Owing to these unique features of the Lake DePue isolate, the JGI has accepted a submission to sequence the genome.

Growth Rates

Duplicate cultures were grown on B3n medium using lactate (20 mM) as a carbon source and sulfate (28 mM) as an electron acceptor Growth was monitored using O.D.$_{600}$ measurements. Values below are listed as maximum growth rates in h^{-1}.

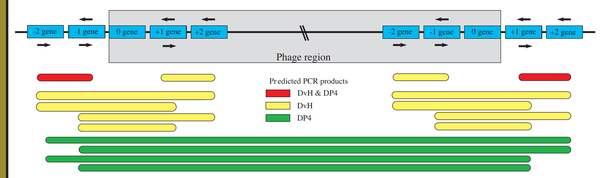
Strain	20 ºC	25 ºC	30 ºC	40 ºC	45 ºC
Hildenborough	0.038	0.084	0.11	0.16	0.14
PT2	0.036	0.067	0.095	0.19	0.06
DP4	0.037	0.087	0.11	0.15	0.13
DP8	0.037	0.087	0.11	0.16	0.15

PFGE

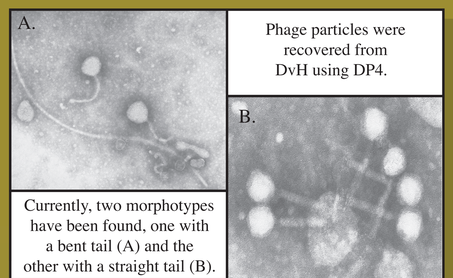


Amplification Schematic of Sequence Spanning the Putative "Phage-Deletion" Sites

Two coding regions (either genes or tRNAs) which were immediately upstream and downstream of both the first and last gene located within the phage-region were selected for primer design. After design, the primers were tested for specificity using DvH genomic DNA and all possible primer combinations at both the beginning and end of the phage-region. The phage-region was bridged using DP4 DNA with all possible combinations of primers. In all cases, PCR product sizes were analyzed on 1% TAE agarose/EtBr gels.



Recovered Phages



Phage particles were recovered from DvH using DP4.

Currently, two morphotypes have been found, one with a bent tail (A) and the other with a straight tail (B).

Conclusions

PHYSIOLOGICAL

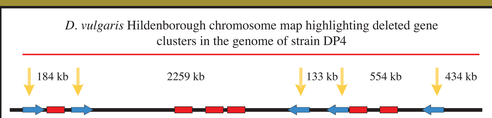
- Morphology and growth rates of isolates are almost identical to DvH.
- No growth on acetate suggests incomplete oxidation pathway similar to DvH.

GENOMIC

- All 9 isolates have identical 16S rRNA, ITS regions and *dsrAB* genes.
- Orthlogous 16S rRNA and *dsrAB* genes of *D. vulgaris* Hildenborough each differ by 1 nucleotide.
- PFGE revealed DP4 has a smaller chromosome and perhaps a larger megaplasmid than DvH.
- Analysis of the DP4 genome using a DvH whole-genome microarray revealed the absence of 295 genes.
- 55 of the deleted genes are annotated as phage or phage-related proteins.
- No additional sequence is present in the three putative "phage-deleted" regions characterized.
- 2 of the 3 regions have direct repeats on each flanking end of the deleted region.
- Novel genetic material and genomic rearrangement present in at least 1 region.
- All putative "phage-deleted" regions of DP4 are flanked immediately upstream or downstream by sequences coding for tRNAs.
- Two phages were recovered by infecting DP4 with supernatant from DvH culture.

Microarray Analysis

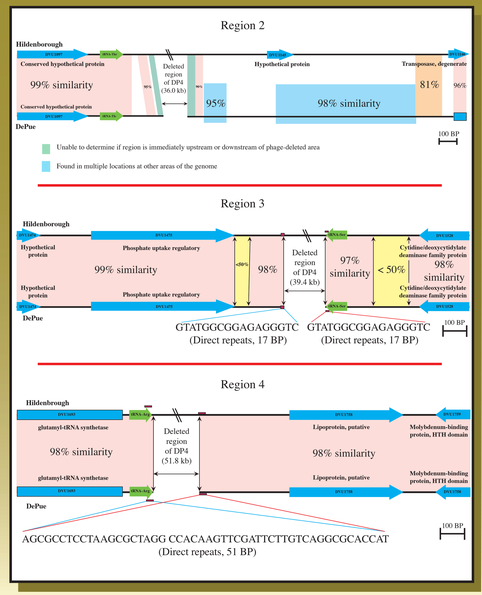
D. vulgaris Hildenborough chromosome map highlighting deleted gene clusters in the genome of strain DP4



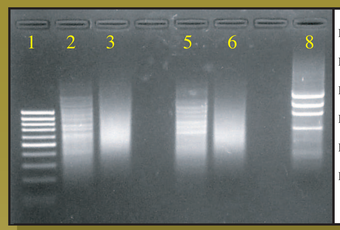
The following table illustrates the distribution of genes missing from the DePue genome. The genes grouped into six distinct regions (see chromosome map above), each approximately 30 - 50 kb in length. While 188 of the 295 genes currently are annotated without a known function other than conserved hypothetical (CH) or hypothetical (H), a large number of phage and phage-related genes were found to be absent.

Deleted region	Length (kb)	# of genes in the following functional categories: CH/H genes	Phage genes: Tail/tail fiber-related	Phage genes: Other	Others
Region 1	36.7	26	3	4	11
Region 2	36.0	24	3	1	10
Region 3	39.4	50	1	2	12
Region 4	51.9	29	7	7	3
Region 5	32.1	31	9	4	10
Region 6	40.7	28	10	4	6
Total	236.8	188	33	22	52

DvH and DP4 Sequence Comparison of Regions Surrounding "Phage-Deletion" Sites



Suppressive Subtractive Hybridization (SSH) of DP4 and DvH

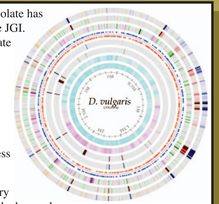


Lane 1 - low range mass ladder
Lane 2 - amplified DP4 SSH
Lane 3 - unamplified DP4 SSH
Lane 5 - amplified DvH SSH
Lane 6 - unamplified DvH SSH
Lane 8 - positive control for SSH

Genome Sequencing

Currently, the genome of the Lake DePue isolate has been accepted for genome sequencing at the JGI. The complete genome sequence of this isolate would enable the VIMSS research effort in the following ways:

- Identify stress response genes and pathways involved in adaptation to a heavy-metal contaminated enivronment.
- Clarify the role of bacteriophages in stress response and adaptation.
- Identify common and disparate regulatory motifs in the isolate and *D. vulgaris* Hildenborough.
- Serve for the development of new genetic tools based on plasmid or phage elements.



Future Work

- Sequencing and annotation of the Lake DePue isolate's genome.
- Comparative analysis of the genome with *D. vulgaris* Hildenborough and other sulfate-reducing bacteria.
- Testing of DePue isolate on various carbon sources, electron donors (acetate, ethanol, sugars, amino acids) and acceptors (sulfur compounds, Fe(III), nitrate, fumarate, other metals).
- Testing of DePue isolate for varying sensitivity when grown in the presence of heavy metals (As, Cd, others).
- DNA/DNA hybridization of Lake DePue isolate for more complete systematic characterization.

Acknowledgements

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